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Loss of maternally-derived human herpesvirus-7 immunity and natural infection in Argentinian infants☆

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Summary
Background: Human herpes virus-7 (HHV-7) infection is widespread throughout the world. No data are available in Argentina about loss of maternally-derived HHV-7 immunity and natural infection.
Objectives: The objective of this study was to characterize the time when children lose maternal antibodies and become susceptible to natural infection.
Methods: Sera from 39 pregnant women and 207 infants between 2 and 29 months of age were tested. Determination of IgG antibodies was made by indirect immunofluorescence.
Results: The seropositive ratio fell in the 2—4 month group (15% seropositive) and increased between 5 months (47% seropositive) and 23 months (67%). Geometric mean titers (GMT) of the infants aged 2—4 months (GMT = 60) were statistically different ($p < 0.0001$, Student's *t*-test) to those from the group of pregnant women (GMT = 83) and those from the other infant groups ($p < 0.001$, least significant difference (LSD) test). The GMT of the groups between 5 and 23 months did not show significant differences whereas those of infants between 24 and 29 months (GMT = 179, 79% seropositive) were different from all the groups studied ($p < 0.0001$, LSD test).
Conclusions: This study shows a significant association between the loss of passive HHV-7 antibody and age. HHV-7 enters the susceptible population at 5 months, leading to the high prevalence of antibodies between 24 and 29 months of age. This study also shows that natural infection by HHV-7 in children during their first years of life follows the infection pattern found in developing countries.

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Introduction

The human herpesvirus-7 (HHV-7) is a lymphotropic virus that was first isolated in 1990 from T-lymphocytes in peripheral blood and saliva of healthy subjects.[1] The isolation of HHV-7 raises questions regarding its prevalence in humans and its potential association with disease. This virus has been associated with exanthem subitum, infectious mononucleosis, and chronic fatigue syndrome among others, even when its specific pathogenic role remains unclear.[2] Primary infection usually occurs in early childhood, often without symptoms, and subsequently the virus persists in the host and is shed in saliva throughout life.[3]

HHV-7, since its isolation, has been found to be closely related to the human herpesvirus-6 (HHV-6) and shares some common antigenic epitopes. Different strategies have been applied to detect anti-HHV-7 antibodies.[4] The immunofluorescence assay for detecting IgG has demonstrated sensitivity and specificity to detect specific antibodies with little cross-reactivity towards HHV-6.[5,6]

Recent studies indicate ubiquity of HHV-7 in most of the populations studied. However, there is little information about the circulation of this virus in developing countries. In Argentina no data are available about infection by HHV-7 and the natural history of the infection remains unknown. This article aims to provide evidence about the loss of maternally-derived immunity and HHV-7 natural infection in Argentinian infants, identifying its particular characteristics.

Materials and methods

Population study

Córdoba is the capital city of the Province of Córdoba with a population of 2 750 000 inhabitants. Sera from 207 healthy infants (aged between 2 and 29 months) and 39 pregnant women collected during routine checkups during the period 1995–96 in the Children's Hospital and in the General Direction of Medical Specialities in Córdoba City were used to investigate the duration of transplacentally-derived HHV-7 immunity. A written consent was obtained from the parents of the children. All infants were born after 36 weeks of gestation weighing >2500 g.

Serologic evaluation

An indirect immunofluorescence assay (IFA) was carried out using multi-well Teflon-coated slides prepared with the lymphoid cell line Supt 1, infected with the SB HHV-7 viral strain. It has previously been determined that the optimal post-infection culture day to be used as a source of viral antigen was day 10 post-infection (data not shown). IFA staining was performed by the standard procedure used in our laboratory. Briefly, 10-fold dilutions of serum samples were incubated with fixed cells for 30 min at 37 °C in a humidified chamber, washed twice with phosphate buffered saline (PBS) for 5 min and incubated for 30 min at 37 °C with fluorescein isothiocyanate-conjugated goat antihuman IgG serum. The slides were mounted with glycerol buffer on coverslips and then examined under a fluorescent microscope.

The highest serum dilution that exhibited a specific fluorescence pattern was considered the endpoint of antibody titration. Titers ≥1:60 were considered significant.

Data analysis

Geometric mean titers (GMT) were calculated only for individuals with detectable antibodies in the following way: the reciprocals of HHV-7 antibody titers were logarithmically converted and the antilogarithm was calculated. To calculate the size of the sample, the prevalence of anti-HHV-7 antibodies was considered to be 70% according to published data,[4] with a confidence level of 95%.[7]

The statistical methodologies were: (a) the Student's *t*-test, to compare the mean of independent samples and (b) the analysis of variance (ANOVA) with one main explanatory variable. The post mean comparison test was later applied using the least significant difference (LSD) test.

Results

The results from maternally-derived HHV-7 antibody titers by age group are summarized in Table 1. The mean ratio of seropositive pregnant women was 48% with a GMT of 83. The seropositive ratio dropped in the 2–4 month group (15% seropositive) and rose between 5 months (47% seropositive) and 23 months (67%). The GMT in infants from 2 to 4 months showed statistically significant differences with the group of pregnant women ($p < 0.0001$, Student's *t*-test). Significant differences were also found in the GMT of the other infant groups (ANOVA, $p < 0.0001$). The group aged between 2 and 4 months was significantly different from all the other groups: with the group aged between 5 and 8 months ($p < 0.001$, LSD test), with the groups aged between 9 and 15 months ($p < 0.002$, LSD test), and with the groups aged between 16 and 29 months ($p < 0.0001$, LSD test). The groups between 5 and 23 months did not show significant differences. The infants aged 24 to 29 months had a GMT of 179 and 79% seropositivity. This group was significantly different from all the other groups of infants ($p < 0.0001$, LSD test) (Figure 1).

Discussion

Seroprevalence of antibodies in pregnant women would indirectly reflect HHV-7 seroprevalence in their infants in early life. Passive immunity protects infants from infection by HHV-7 during the first months of life.

Table 1 HHV-7 antibodies in infants according to age group

Age (months)	Number	GMT of seropositive children	Seropositive % (95% CI)
2–4	26	60	15 (9–21)
5–8	27	110	47 (41–53)
9–11	48	102	43 (37–49)
12–15	28	83	53 (47–59)
16–19	26	102	65 (61–71)
20–23	28	96	67 (73–61)
24–29	24	179	79 (73–85)

GMT, geometric mean titers. CI, confidence interval.

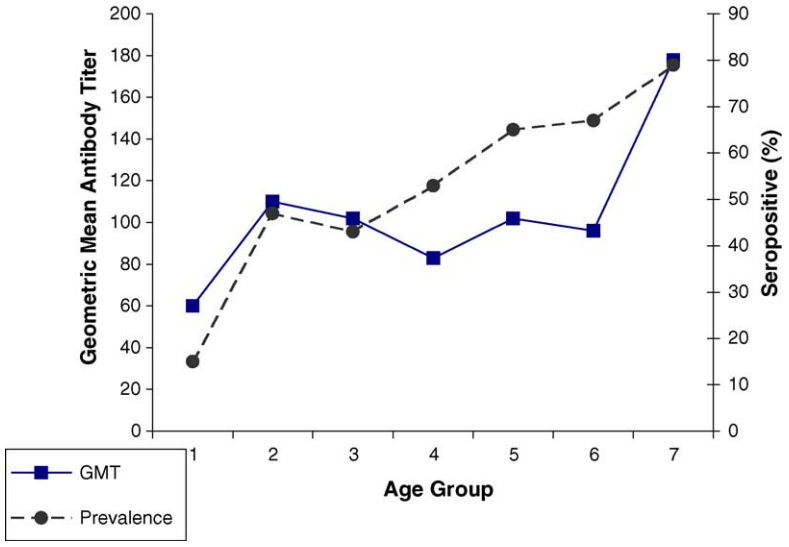

Figure 1 Prevalence of HHV-7 antibodies and the geometric mean titers of HHV-7 detectable antibodies in infants by age group: (1) 2—4 months, (2) 5—8 months, (3) 9—11 months, (4) 12—15 months, (5) 16—19 months, (6) 20—23 months, and (7) 24—29 months.

The diminution of the GMT in infants aged 2 to 4 months with respect to the values in pregnant women would derive from the catabolism of maternal IgG. Although there has been no direct proof of protection by maternal HHV-7 antibodies, this study shows that HHV-7 infection increases after the fifth month of life, suggesting a protective role for the maternal antibody during the first four months of life. If so, the prevalence and antibody titers observed in 2 to 4 month-old infants would be as a result of transplacental antibodies and/or antibodies resulting from primary infection.

HHV-7 primary infection took place around 5—8 months of age, and the immune system received a boost at a later time, around 2 years of age.

In a previous report we have demonstrated that HHV-6 enters into the susceptible population between 13 and 15 months of age.[8] In the present report the results are surprising as primary infection with HHV-7 occurs earlier than with HHV-6. The explanation is very unlikely to lie in antibody cross-reactivity between HHV-7 and HHV-6 as measured by immunofluorescence tests. The limited cross-reactivity between naturally induced human antibodies against the two viruses has been taken into account. According to several researchers,[2,9] in the sera with low IgG titers for HHV-7 (1:30), a cross-reactivity with HHV-6 is probable. For this reason in our study the serum starting at dilution 1:60 was considered significant.

In order to determine the age of primary acquisition of HHV-7, we referred to Oliveira et al.,[10] who have reported 71% of HHV-7 primary infection in children less than 1 year old in a tropical region of Brazil. The onset of HHV-7 antibodies within the first year has also been reported in Hungarian children.[11] On the other hand, according to some authors,[2,9,12] HHV-7 infection usually occurs later, being gradually acquired over the first five or six years of life with about 65% of children infected by the age of three. Furthermore, Yoshikawa et al.[13] have reported that in Japan, the children reached the highest level (60%) at 11—13 years of age. On account of these findings, it seems probable that epidemiology of HHV-7 could have two different patterns, one of early primary infection in the developing world and another one of late primary infection in the industrial or developed nations.

This study shows that natural infection by HHV-7 in children during their first years of life follows the infection pattern found in the developing countries. This constitutes a starting point in the examination of the pathogenic role of this virus in different clinical entities.

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Conflict of interest: No conflict of interest to declare.

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